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# Effects of protein sources with differing ruminal degradation characteristics on nutrient digestibilities and flows through various segments of the gastrointestinal tract of non-lactating Holstein cows

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EFFECTS OF PROTEIN SOURCES WITH DIFFERING RUMINAL DEGRADATION  
CHARACTERISTICS ON NUTRIENT DIGESTIBILITIES AND FLOWS THROUGH  
VARIOUS SEGMENTS OF THE GASTROINTESTINAL TRACT OF NON-  
LACTATING HOLSTEIN COWS

A Thesis

Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
In partial fulfillment of the  
Requirements for the degree of  
Master of Science

in

The Interdepartmental Program in Animal and Dairy Sciences

by

Taylor W. Braud  
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## **ABSTRACT**

Six non-lactating Holstein cows with ruminal and duodenal cannulas were used in a replicated 3x3 Latin square design experiment to investigate the effects of supplemental protein source on ruminal and total tract nutrient digestibility. Supplemental protein was provided from soybean meal (SBM), expeller processed soybean meal (EXP), or menhaden fish meal (FM). Basal diets consisted of (DM basis) 33% corn silage, 20% bermudagrass hay, 27% ground corn, and 2% minerals and vitamins. Supplemental protein was provided as (DM % of total diet) 18% soybean meal (SOY), 17% soybean meal and 1% fish meal (FM), or 12% soybean meal and 6% expeller processed soybean meal (EXP). Period length was 14 d. Flow of digesta was estimated using  $\text{Cr}_2\text{O}_3$  as an external marker. Due to factors unrelated to the trial, one cow died during the first period of the study and was not replaced. Dry matter intake averaged 9.5 kg/d and was not affected by treatment. Apparent ruminal dry matter digestibility was not affected by source of supplemental protein. Source of supplemental protein did not affect apparent total tract dry matter digestibility. Feeding low ruminal degradable protein to non-lactating cows resulted in no appreciable impact on feed intake or apparent diet digestibility.

## CHAPTER 1: REVIEW OF LITERATURE

### Introduction

Protein supplementation strategies for feeding dairy cattle have evolved over time. Until recently, when discussing ruminant protein nutrition, crude protein was the unit of choice. Crude protein is defined for feedstuffs as nitrogen content  $\times 6.25$ . For many years, crude protein was used in formulating diets for lactating dairy cows because little was known of the response to dietary protein of varying quality, and many researchers postulated that the high quality microbial protein synthesized in the rumen would complement deficiencies in the quality of dietary protein that escaped ruminal fermentation (Cheeke, 2005). In the 1980's, dairy nutritionists became aware that microbial protein could not supply enough amino acids to meet the requirements of a high-producing dairy cow, and the ruminally undegradable protein/ruminally degradable protein system began to get more usage (Cheeke, 2005). Although this system worked well in the field, it was not able to fully optimize amino acid nutrition of the cow because it still treated the rumen as a "black box" that one protein went into and a different protein came out (Cheeke, 2005). As computer technology advanced, so did the ability of the nutritionist to predict flow of proteins and amino acids at the small intestine. This allows for better fine-tuning of amino acid nutrition in the modern dairy ration (Cheeke, 2005); however, errors in the computer systems still exist and further improvements need to be made (Bateman *et al.*, 2005). Advances in the understanding of protein nutrition of the dairy cow made between the 1989 and 2001 editions of the Nutrient Requirements of Dairy Cattle publications were enormous. In the 1989 edition, protein requirements were based on microbial crude protein (MCP) and undegradable intake protein (NRC, 1989).

The term undegradable intake protein has been replaced by the term ruminally undegradable protein (RUP) which is considered a better descriptor of the behavior of those proteins (NRC, 2001). The MCP was predicted based on  $NE_L$ , and RUP was predicted based on constants for the degradability of proteins in feeds. Time and experience have proven that neither of these assumptions are true. Research has shown that microbial protein synthesis is better related to organic matter fermentation in the rumen than to  $NE_L$  intake, which is reflective of both ruminal fermentation and postruminal digestion. The current publication (NRC, 2001) predicts MCP production based on total digestible nutrient intake but requires that ruminally degradable protein is adequate for optimal fermentation. It has also been recognized that RUP values for a feed cannot be static across all feeding situations and diets (Bateman *et al.*, 2005). The current NRC publication (2001) attempted to predict the RUP content of feeds based on their fermentability in a given diet at a given dry matter intake. As with energy values, these changes appear to better explain variation in milk production.

Ruminants have the unique ability to convert feedstuffs not suitable for human consumption into products such as meat and milk through a synergistic relationship with the microbes of the rumen (Cheeke, 2005). The rumen microbes can utilize feeds low in protein and energy for growth and reproduction. These microorganisms travel out of the rumen to the abomasum and then to the small intestines, where they are used by the ruminant as a protein supply. This microbial protein supply has an amino acid profile capable of meeting the maintenance requirements of most mature cattle (Church, 1988). Proteins reaching the small intestine of the ruminant are derived from three sources: (a) dietary protein which has escaped breakdown by rumen microbes; (b) protein contained



in bacterial and protozoal cells which flow out of the rumen and (c) endogenous proteins contained in sloughed cells and secretions into abomasum and intestine (Cheeke, 2005). Postruminal protein digestion and amino acid absorption are expected to occur in fashions similar to those observed in non-ruminants. Ruminants rely upon the same complement of pancreatic and intestinal proteases to affect breakdown of protein as do non-ruminants and absorb amino acids and small peptides by similar mechanisms (Church, 1988). In most instances, when protein digestibility is measured, it is referred to as apparent protein digestibility. True protein digestibility includes a correction for endogenous protein or nitrogen, also called metabolic fecal nitrogen. Endogenous protein is associated with protein that did not come directly from the diet but is secreted into the gut as digestive enzymes and sloughed-off cells of the gut mucosa. The lining of the gut undergoes continual replacement with new cells and the sloughing off of old cells, which contain protein that is excreted in the feces. For most purposes, apparent protein digestibility values are adequate (Cheeke, 2005).

Previous work from our lab (Richardel, 2004) evaluated soybean meal and fish meal as protein supplements for growing Holstein steer calves. No differences were observed for growth of the calves although calves fed soybean meal tended to have higher plasma growth hormone concentrations. Levels of inclusion for fish meal in the previous project were kept low to minimize feed refusals by the calves. It may be possible that larger inclusion rates or other sources of supplemental protein, such as expeller processed soybean meal, would alter nutrient flow at the small intestine to a greater extent than small amounts of fish meal. Knowledge of the nutrient digestibility

and flow through various segments of the gastrointestinal tract when similar changes in protein source would be helpful for planning future work in this area.

### **Objective**

The objective of this review will focus on the impact of altering ruminal protein digestibility on nutrient flow and digestibility in cattle.

### **Protein Chemistry**

The wide variety of 3-dimensional protein structures corresponds to the diversity of functions proteins fulfill. Protein structure is organized hierarchically from primary structure to quaternary structure (Nelson and Cox, 2005). Higher-level structures are motifs and domains. Above all, the wide variety of conformations is due to the huge amount of different sequences of amino acid residues. The primary structure is the sequence of residues in the polypeptide chain. The order of individual amino acids joined by covalent peptide bonds determines the primary structure of a protein.

Secondary structure is a local regularly occurring structure in proteins and is mainly formed through hydrogen bonds between backbone atoms. The polypeptide chain is pulled into the helical shape by weak hydrogen bonds. Random coils, loops or turns don't have a stable secondary structure. There are two types of stable secondary structures:

Alpha-helices and beta-sheets. Alpha-helices and beta-sheets are preferably located at the core of the protein, whereas loops prefer to reside in outer regions (Nelson and Cox, 2005). Tertiary structure describes the packing of alpha-helices, beta-sheets and random coils with respect to each other on the level of one whole polypeptide chain. The whole polypeptide chain undergoes further folding and coiling in response to the chemical bonds and interactions between individual amino acids of the protein. Quaternary

structure only exists, if there is more than one polypeptide chain present in a complex protein. Then quaternary structure describes the spatial organization of the chains. These chains are also held together by chemical bonds (Nelson and Cox, 2005).

### **Ruminal Measures**

Protein solubility has been used as a proxy for ruminal protein degradability but the two measurements are not the same. Protein solubility can be measured in either water or buffer. The current NRC (2001) publication for dairy cattle uses a borate phosphate buffer for estimation of the soluble fraction of feed proteins. In many feeding models, soluble protein is represented as fraction A and is considered to be instantly available in the rumen for microbial protein synthesis. Optimal utilization of soluble protein is dependant upon having adequate rapidly available carbohydrate present for fermentation. Sources of NPN such as urea, ammonia, or free amino acids are assumed to be 100% soluble (Van Soest, 1994).

The in situ (in sacco) procedure is the technique of suspending a polyester bag filled with test components in the rumen through a fistula for a specific time period (Owens and Goetsch, 1993; Van Soest, 1994). Ruminal microorganisms, ruminal fluid, and end products of digestion can be flushed in and out of the cloth bag via small pores. The portion of the test component that disappears from the bag is considered to have been digested. The in situ system provides the advantages of: 1) rapid measuring of the digestibility of forages and proteins, 2) digesting test components in a real ruminal environment, and 3) avoiding rapid changing in microbial types, which may occur in batch culture systems (Owens and Goetsch, 1993; Van Soest, 1994). The in situ system also has disadvantages that include: 1) accumulation of lignified materials or microbes in

the bag, 2) low or even negative digestibility estimates, and 3) efflux of small particles without being digested (Owens and Goetsch, 1993). An improved method by Udén *et al.* (1974) and Van Hellen and Ellis (1997) used specific pore sizes and controlled the ratio of sample weight to surface area of the bag and this has resulted in commercial production of Dacron® bags for in situ evaluations.

An in vitro system can be defined as a system in which ruminal microbes are incubated with feedstuffs or test components in a batch, a continuous, or a discontinuous culture. The sequence of all in vitro rumen procedures is an anaerobic fermentation of a sample substrate with medium and filtered rumen liquid followed by an end point measurement (Van Soest, 1994). The medium is generally a buffer solution simulating ruminant saliva. Compared to in vivo systems, in vitro systems are generally lower in cost, faster, and more repeatable (Owens and Goetsch, 1993). There are two styles of in vitro systems that have been studied and used extensively. They include the batch incubation and the continuous culture system.

The batch incubation, as described by Tilley and Terry (1963), is a procedure commonly used in forage evaluation. The method involves two stages: 24 or 48 hour digestion of test feedstuffs with buffered ruminal fluid followed by 48 hour digestion with pepsin-HCl to solubilize proteins (Owens and Goetsch, 1993; Van Soest, 1994). The Tilley-Terry system only needs small amounts of test feedstuffs, and this system can be run rapidly compared to in vivo evaluations. In vitro dry matter disappearance can be measured by this system and is correlated with in vivo digestibility (Owens and Goetsch, 1993). A modified procedure has been developed to shorten and simplify the original procedure. It includes substitution of buffered ruminal fluid with a high-phosphate

buffer, which acidifies directly and supplies pepsin during the second stage, and decreases pepsin digestion time to one day (Van Soest, 1994). In general, the Tilley-Terry system has been a relatively accurate procedure for appraising digestibility in the laboratory when applied to reasonable-quality feedstuffs without supplements (Van Soest, 1994).

Compared to the Tilley-Terry system, the continuous culture system has been studied more extensively not only for nutrient digestibility but also ruminal microorganism metabolism. The operating conditions of the continuous culture system are more similar to the in vivo environment than closed vessel incubation. The continuous culture system described by Slyter *et al.* (1964) had been reported to maintain similar bacterial numbers as in the rumen (Slyter and Putnam, 1967), but protozoa numbers were decreased markedly compared to ruminal protozoa numbers. Other studies (Abe and Kumeno, 1973) also reported decreased protozoa numbers using a similar continuous culture system. The dual flow continuous culture system described by Hoover *et al.* (1976) was designed to stimulate the differential dilution rates of liquids and solids that occur in the rumen and has been reported to improve the maintenance of protozoa numbers compared to the single overflow system. Hannah *et al.* (1986) used a modified dual flow continuous culture system to evaluate the feasibility of in vitro fermentation data compared to in vivo data. They (Hannah *et al.*, 1986) reported similar true OM digestibility and crude protein and AA degradability in both the in vitro and in vivo systems. This indicated that the dual flow continuous culture system could provide reasonable estimates of ruminal fermentation. However, results of in vitro fermentation should be evaluated in vivo. Concepts which are ineffective in vitro should be ineffective

in vivo, but concepts which are effective in vitro may not be effective in vivo because of microbial and animal adaptations and changes in the ruminal environment (Owens and Goetsch, 1993)

The term in vivo normally refers to research performed on whole organisms. In vitro research has the advantage over in vivo research that there are fewer variables which can confound an experiment, and that if an experiment effect is subtle the result will be clearly visible (Van Soest, 1994). In vivo research has the advantage, over in vitro research, that the experimental system is a more complex biological system. This means that in vivo research will likely give a better indication of what will happen in a population when a compound is administered to or a procedure is applied to a live animal in production settings (Van Soest, 1994).

### **Microbial Protein Synthesis**

Microbial protein is an end product of the ruminal fermentation of carbohydrates. Microbial protein that reaches the duodenum contributes the greatest amount of protein for the ruminant animal (Firkins, 1996). Schwab (1996) indicated that ruminally synthesized microbial protein supplies 50% or more of absorbed AA when rations are balanced properly. Microbial protein is considered to be a consistent (Schwab, 1996) and high quality protein source with a balanced AA profile (Clark *et al.*, 1992) and is also relatively less expensive to produce compared to other protein sources. Ruminal microorganisms do not have AA requirements per se but do have requirements for branched-chain fatty acids and  $\text{NH}_3$  for microbial protein synthesis. This is because cross feeding of microorganisms within the rumen can provide adequate preformed AA for microbial growth. However, ruminal microorganisms have been shown to increase their

growth rate when provided with preformed AA either as free AA or as peptides (Cotta and Russell, 1982; Jones *et al.*, 1998)

Microbial protein is assumed to have the same intestinal digestibility as RUP at 80 to 90% (Schwab, 1996). The biological value of microbial protein ranges from 66 to 87%, and microbial protein is approximately 80% AA (Owens and Zinn, 1993).

Microbial protein synthesis is limited by ATP production and depends largely on the ruminal availability of N and carbohydrates (NRC, 2001) that can provide carbon skeletons and energy (Stern *et al.*, 1994). Therefore, synchronization of ruminally degradable carbohydrates with RDP is extremely important for optimizing microbial protein synthesis (Firkins, 1996; Nocek and Russell, 1988; NRC, 2001; Stern *et al.*, 1994). Factors that influence ruminal fermentation and microbial protein synthesis include amount and type of supplemental protein, carbohydrate sources and availability in the rumen, and ruminal pH (Bateman *et al.*, 1999).

### **Ruminally Undegradable Protein**

Any deficiency in quality of the AA profile provided solely by microbial protein can be corrected by feeding supplemental sources of RUP. These types of protein sources resist degradation by the ruminal microbes and reach the abomasum basically unaltered (Merchen and Titgemeyer, 1992). Protein supplements that are high in RUP and are commonly used in ruminant diets are fish meal (FM), feather meal, blood meal, corn gluten meal, distillers dried grains, distillers dried grains with solubles, brewers dried grains, brewers wet grain, roasted soybeans, and heat treated soybean meal (Santos *et al.*, 1998). Because they resist degradation, the AA profile of these feeds that is

available for absorption is determined predominantly by the AA profile of the original RUP supplement.

Supplementation with RUP can improve animal performance by altering the protein to energy ratio of potentially absorbable nutrients. Beef steers fed ad libitum grass silage and supplemented with FM had increased average daily gains compared to those supplemented with barley (Veira *et al.*, 1988). According to those researchers, improvement of the protein to energy ratio enhanced the animal's ability to deposit protein and thus increased the growth of the beef steers. Bethard *et al.* (1997) reported that high RUP fed in the form of blood meal improved the feed efficiency of growing Holstein heifers as well as apparent total digestible nutrient efficiency. Donaldson *et al.* (1991) fed RUP in the form of FM and distiller's dried grains to abomasally cannulated beef steers. These researchers reported greater dry matter intake and increased abomasal crude protein and non-ammonia nitrogen flows compared to corn supplementation. This increase allowed an improvement in the AA balance available for absorption by the small intestine.

More importantly, RUP supplementation improves the quality and quantity of AA reaching the small intestine for absorption (Merchen and Titgemeyer, 1992). This change in AA profile could cause a growth response to occur. Tomlinson *et al.* (1997) reported growth responses when blood meal was added to the diets of Holstein heifers. In that study, heifers had improved feed efficiency as well as increased gains when RUP percentage increased in the diet. Zerbini and Polan (1985) reported improved average daily gains when FM replaced soybean meal in diets fed to Holstein bull calves. Gill and Beever (1982) fed FM and ryegrass silage to Holstein steers and reported increased flow



of AA to the small intestine with an increase in the amounts of specific AA (methionine, lysine, arginine, and glutamine). These researchers also reported an increase in growth in the steers fed FM.

Fish meal is an excellent source of RUP and has been shown to cause and increase in growth when supplemented to the diets of growing ruminants. Addition of FM to formic-acid-treated grass silage fed to weaned steers resulted in significantly greater gains than silage alone or silage supplemented with barley (Veira *et al.* 1985). Additional work by Veira *et al.* (1988) resulted in increased average daily gains in beef steers fed ad libitum grass silage supplemented with FM compared to beef steers supplemented with barely. Addition of FM to the diets of beef calves increased average daily gain, feed efficiency and plasma AA profiles when compared to control diets that were supplemented with SBM only (Davenport *et al.*, 1991).

### **Ruminally Degradable Protein**

Ruminally degradable protein is used by the ruminal microbes when they convert feed nutrients to microbial protein rather than by the animal itself. Ruminally degraded protein can come in the form of NPN or true protein. It supplies the rumen microbes with ammonia, amino acids, and peptides. These nutrients are used by the microbes to support microbial fermentation. A deficiency in RDP would result in reduced carbohydrate digestion, VFA and microbial protein production. This would decrease animal performance. In a study conducted to determine RDP requirement of finishing steers fed steam-flaked corn (Cooper *et al.*, 2002) it was reported that as dietary RDP increased feed to gain ratio and average daily gain increased quadratically. Dry matter intake also increased quadratically in response to increased RDP. Little research has been done with

supplementation of RDP. Feeding RDP below the requirements for maximal rumen microbial growth can compromise microbial protein production, ruminal digestion, and energy and protein availability to the cow (Stokes *et al.*, 1991; Clark *et al.*, 1992). Therefore, determining the level of dietary RDP required for optimum N utilization by ruminal microbes would allow for reductions in the dietary CP levels without compromising milk production, thereby increasing feed efficiency and reducing feed costs and N losses to the environment. Moreover, quantitative in vivo estimates of RDP requirements will be useful for the accuracy of the predictions of ration formulation models.

Ruminants can utilize NPN as well as dietary protein. Based on the rate and extent of ruminal degradation, NPN in feeds and supplements, such as urea and ammonium salts, are considered to be completely degraded in the rumen (NRC, 2001). Substitution of NPN for natural protein always decreases the cost of dietary protein, but production responses seldom increase with the addition of NPN (Owens and Zinn, 1993). There are some important rules that need to be kept in mind when using NPN (Owens and Zinn, 1993). First, NPN only supplies  $\text{NH}_3$  for ruminal bacteria but does not supply energy and minerals as do plant and animal protein sources. Second, high producing dairy cattle utilize natural protein more efficiently than NPN because the protein requirements for dairy cattle during early lactation are greater than the protein requirements for the ruminal microorganisms. Third, NPN is degraded faster and more completely than natural protein sources in the rumen. If most of the dietary RDP is supplied by NPN, too much readily soluble N is presented to the ruminal microorganisms, and excess N may leave the rumen as  $\text{NH}_3$  before it can be incorporated

into microbial protein. But, if most of the dietary RDP is supplied by natural proteins, excess protein may escape or bypass ruminal degradation and still be available to the host at the lower gut. This is especially true during the early stages of lactation. Fourth, NPN is recommended to be used only during mid or late lactation since protein requirements of the host animal are lower during this time.

### **Thesis Objective**

The objective of the experiment described in this thesis was to determine if substituting protein sources with decreased ruminal degradability for soybean meal in diets fed to non-lactating Holstein cows would alter nutrient digestibility and flow through various segments of the gastrointestinal tract.

## CHAPTER 2: MATERIALS AND METHODS

Six non-lactating Holstein cows (average BWT 642 kg) were surgically fitted with cannulas in their rumen and proximal duodenum by the School of Veterinary Medicine at Louisiana State University under protocols approved by the LSU Agricultural Center's Institutional Animal Care and Use Committee and allowed to recover. These cows were then used in a replicated  $3 \times 3$  Latin square designed experiment. Duodenal cannulas were a simple – T gutter type design made from soft plastic material (B. Hess, Univ. of Wyoming). Ruminal cannulas were from a commercial source (Bar-Diamond, Inc., Parma, ID). Due to factors unrelated to the trial, one cow died during the first period of the study and was not replaced.

Supplemental protein was provided by soybean meal (**SOY**), expeller processed soybean meal (**EXP**; West Central Cooperative), or menhaden fish meal (**FM**). Ingredient and chemical composition of experimental diets is presented in Table 1. Basal diets consisted of (DM basis) 33% corn silage, 20% bermudagrass hay, 27% ground corn, and 2% minerals and vitamins. Supplemental protein was provided as (DM % of total diet) 18% soybean meal (SOY), 17% soybean meal and 1% fish meal (FM), or 12% soybean meal and 6% expeller processed soybean meal (EXP). Diets were offered once daily in amounts adequate to allow for 10% (as offered) feed refusals.

Period length for this experiment was 14 days. During each period, cows were allowed to adapt to diets for the first 10 days and then data were collected. Two gelatin capsules containing 10 g of  $\text{Cr}_2\text{O}_3$  were placed in the rumen once daily beginning on day 10 of each period to be used as an external marker of digesta flow. Samples of duodenal contents, ruminal contents, and feces were collected starting at 0800 h on

**Table 1.** Ingredient and chemical composition of experimental diets

Ingredient	SOY	EXP	FM
	% of DM		
Corn silage	32.91	32.91	33.08
Bermudagrass hay	20.37	20.37	20.48
Ground corn	26.53	26.53	26.66
Soybean meal	18.32	12.21	16.84
Expeller processed soybean meal <sup>1</sup>	0	6.11	0
Fish meal	0	0	1.09
Mineral and vitamin premix	1.87	1.87	1.85
Item			
DM, %	50.21	52.80	50.73
	% of DM		
OM	90.71	91.70	91.89
CP	12.36	12.20	13.35
ADF	39.19	41.54	37.54
NDF	82.42	85.04	86.37
RUP <sup>2</sup>	5.4	6.1	5.8

<sup>1</sup> SoyPLUS, West Central Cooperative., Ralston IA.

<sup>2</sup> Calculated using the model of NRC (2001) at observed DMI.

day 12 of each period and every 3 h thereafter with sampling time being advanced 2 h on each day. Therefore, samples were collected that represent each hour on the 24 h clock.

Diets and refusals were sampled on each sampling day of each period.

Duodenal samples were collected by opening the cannula and allowing contents to flow freely to clear the cannula of sedimentary material. After approximately 100 mL of contents had flowed from the cannula, 250 mL samples were collected then composited within each cow and period observational unit and stored frozen (-20 ° C) until analysis. Samples of ruminal solids were collected by hand from the ruminal mat directly in front of the cannula. Samples were composited within each cow period observation and stored frozen until analysis. Fecal grab samples were collected from immediately inside the rectum and composited within each cow period observation. At the completion of each period, samples were thawed and further prepared.

Rumen composite samples were thawed at room temperature for analysis. A modified isolation procedure adapted from Zinn and Owens (1980) and Steinhour et al. (1982) was used to isolate bacteria from the rumen samples. The entire sample contents were blended in a Waring commercial blender at low speed for 5 min. to separate the bacteria from the feed particles. Sufficient quantities of 0.9% saline solution were added as needed to reduce the viscosity of the blended fluid. The blended fluid was strained through four layers of cheesecloth and solids were discarded. Strained fluid was centrifuged at 500x g for 5 min to remove the remaining feed particles and protozoa. The supernatant was decanted, and the pellet was discarded. The supernatant was re-centrifuged at 20,000x g for 20 min. The supernatant from this second centrifugation step was decanted and discarded. The pellet was re-suspended in 0.9% saline solution and re-

centrifuged at 20,000x g for 20 min. Again, the supernatant was decanted and discarded. This step was repeated once more prior to harvesting the bacterial pellet, which was stored frozen (-20 °C) until later analysis. Bacterial pellets were then thawed at room temperature. Effluent pellets and isolated bacteria were dried at 55°C overnight, and dry weight was recorded. Oven dried effluent pellets and bacteria were ground by hand. Effluent pellets and bacteria were then dried at 105°C and analyzed for their contents of ash, purine, and N. Ash was analyzed according to an AOAC procedure (1980). Purines were measured to the procedure of Zinn and Owens (1986). Concentrations of purines in bacteria and rumen effluent were measured in order to separate N into microbial and dietary fractions (Zinn and Owens, 1986). Contents of N in effluent and bacterial pellets were analyzed as Kjeldahl N.

Fecal and duodenal samples were dried to a constant weight at 40° C in a forced air oven. Content of Cr in samples of duodenal and fecal contents were determined by atomic absorption spectrophotometry (Williams *et al.*, 1962) and used to calculate nutrient flows. Digesta flow was calculated by dividing Cr dose per day by Cr concentration in the sample (Cheeke, 2005). Estimated flow of digesta was multiplied by concentrations of nutrients in the digesta to calculate nutrient flows. Calculated nutrient flows were used to calculate both apparent and true digestibility. Apparent digestibility does not account for endogenous secretions or bacterial synthesis [apparent digestion = nutrient intake – nutrient output]. True digestibility accounts for what is actually digested and absorbed by the animal in addition to endogenous secretions [true digestion = intake – (output – bacteria)].

All data were analyzed using a mixed model (Littell *et al.*, 1998) and SAS (SAS Institute Inc., 1990). The model included terms for the fixed effect of treatment and the random terms of cow and period. Significance was declared at  $P < 0.05$  and trends when  $P \geq 0.05$  and  $< 0.1$ . When a significant F was observed, Fisher's LSD was used to separate treatment means. Because of the missing data all standard errors are not equal. Therefore, data are presented as least squares means along with the largest standard error.



## CHAPTER 3: RESULTS

### Diet Analysis

Chemical analyses of the experimental diets are reported in Table 1. Content of DM, OM, CP, ADF, and NDF were similar across all experimental diets (Table 1). However, the three diets were low or marginal in CP content at 12% of DM and high in fiber (80% NDF and 40% ADF) compared to NRC 2001 recommendations.

### Nutrient Digestion and Digestibility

Least squares means for intake and digestibility of DM at various locations in the gastrointestinal tract are presented in Table 2. When FM was the source of supplemental protein, DMI was greater ( $P < 0.05$ ) than when SOY or EXP diets were used as the source of supplemental protein. However, intakes of diets with SOY or EXP as the source of supplemental protein were not significantly different from one another ( $P > 0.1$ ). No significant differences were found for apparent ruminal or total tract DM digestibility of the three diets. However, there was a tendency for more DM to be apparently digested in the rumen when FM was the source of supplemental protein. Similarly, there were no differences in true ruminal digestibility of DM among the three diets, but there was a tendency ( $P < 0.1$ ) for more DM to be truly digested in the rumen when FM was the source of supplemental protein.

Least squares means for intake and digestibility of OM at various locations in the gastrointestinal tract are listed in Table 2. When FM was the source of supplemental protein, OMI was greater ( $P < 0.05$ ) than when SOY or EXP diets were used as the source of supplemental protein. However, intakes of diets with SOY or EXP as the

**Table 2.** Least squares means for intake and digestibility of DM and OM at various locations in the gastrointestinal tract of cows fed diets with soybean meal (SOY), expeller processed soybean meal (EXP), or fish meal (FM) as the source of supplemental protein.

Item	SOY	EXP	FM	SEM
DM				
Intake, kg/d <sup>A</sup>	10.8 <sup>b</sup>	10.9 <sup>b</sup>	12.3 <sup>a</sup>	0.78
ADR <sup>1</sup>				
Kg/d <sup>B</sup>	6.2	6.0	7.5	0.68
%	56.1	57.3	61.2	2.8
ADTT <sup>2</sup>				
Kg/d <sup>B</sup>	8.2	8.2	9.7	0.69
%	76.0	74.3	79.3	2.1
TDR <sup>3</sup>				
Kg/d <sup>B</sup>	7.9	7.7	9.1	0.87
%	72.0	73.5	73.3	2.9
OM				
Intake, kg/d <sup>A</sup>	9.8 <sup>b</sup>	10.0 <sup>b</sup>	11.3 <sup>a</sup>	0.74
ADR <sup>1</sup>				
Kg/d <sup>B</sup>	6.0	5.9	7.4	0.69
%	60.7	61.5	66.0	2.8
ADTT <sup>2</sup>				
Kg/d <sup>B</sup>	7.7	7.8	9.1	0.67
%	78.6	77.1	81.2	2.0
TDR				
Kg/d	7.6	7.4	8.8	0.86
%	76.2	77.1	77.7	3.2

<sup>1</sup> Apparently digested in the rumen.

<sup>2</sup> Apparently digested in the total gastrointestinal tract.

<sup>3</sup> Truly digested in the rumen.

<sup>A</sup> Treatments differ ( $P < 0.05$ ).

<sup>B</sup> Tendency for treatments to differ ( $P \geq 0.05$  and  $< 0.1$ ).

<sup>a,b</sup> Means within the same row with differing superscripts differ ( $P < 0.05$ ).

source of supplemental protein were not significantly different ( $P > 0.1$ ). No significant differences were found for apparent ruminal or total tract OM digestibility of the three diets. However, there was a tendency for more OM to be apparently digested in the rumen when FM was the source of supplemental protein. There were no differences in true ruminal digestibility of OM among the three diets nor were there any differences among the amount of OM truly digested in the rumen.

Least squares means for flow of NDF and ADF through various segments of the gastrointestinal tract are presented in Table 3. There was tendency for more NDF to be apparently digested in the rumen when FM was the source of supplemental protein when compared to EXP. However, apparent NDF digestion in the rumen when SOY was fed was not different from FM or EXP. Apparent digestion of ADF in the rumen and total gastrointestinal tract was not affected ( $P > 0.1$ ) by source of supplemental protein. Similarly, apparent digestibility of ADF in the rumen and total gastrointestinal tract was not affected ( $P > 0.1$ ) by source of supplemental protein.

Least squares means for intakes and digestibility of N in various segments of the gastrointestinal tract of cows are presented in Table 4. Intake of N in cows fed SOY was significantly lower than cows fed FM ( $P < 0.05$ ). N intake for cows fed EXP was intermediate to those fed FM and SOY. There were no differences in apparent ruminal or total tract digestibility or digestion of N due to source of supplemental protein. No differences were observed due to source of supplemental protein for nitrogen apparently digested postruminally.

**Table 3.** Least squares means for digestibility and flow of NDF and ADF through various segments of the gastrointestinal tract of cows fed diets with soybean meal (SOY), expeller processed soybean meal (EXP), or fish meal (FM) as the source of supplemental protein

Item	SOY	EXP	FM	SEM
NDF				
ADR <sup>1</sup>				
kg/d <sup>A</sup>	6.2	5.5	7.5	0.97
%	65.3	64.2	70.8	0.87
ADTT <sup>2</sup>				
kg/d	7.4	7.4	8.6	0.87
%	79.3	78.8	82.7	2.1
ADF				
ADR <sup>1</sup>				
kg/d	2.7	3.2	3.0	0.45
%	65.7	69.3	72.3	4.0
ADTT <sup>2</sup>				
kg/d	2.9	3.3	3.1	0.44
%	69.1	71.3	74.0	3.5

<sup>1</sup> Apparently digested in the rumen.

<sup>2</sup> Apparently digested in the total gastrointestinal tract.

<sup>A</sup> Tendency for treatments to differ ( $P \geq 0.05$  and  $< 0.1$ ).

**Table 4.** Least squares means for intakes and digestibility of N in various segments of the gastrointestinal tract of cows fed diets with soybean meal (SOY), expeller processed soybean meal (EXP), or fish meal (FM) as the source of supplemental protein

Item	SOY	EXP	FM	SEM
N				
Intake, g/d <sup>A</sup>	220.2 <sup>b</sup>	230.4 <sup>ab</sup>	249.6 <sup>a</sup>	36.2
ADR <sup>1</sup>				
g/d	64.2	55.5	38.9	33.1
%	23.7	20.0	14.2	12.9
ADTT <sup>2</sup>				
g/d	144.0	150.7	162.4	38.2
%	62.4	62.4	67.6	6.7
ADPR <sup>3</sup>				
g/d	79.6	96.5	122.0	20.6
% of intake	36.1	41.9	48.9	13.5
% of duodenal passage	51.0	55.2	57.9	12.4

<sup>1</sup> Apparently digested in the rumen.

<sup>2</sup> Apparently digested in the total gastrointestinal tract.

<sup>3</sup> Apparently digested postruminally.

<sup>A</sup> Treatments differ ( $P < 0.05$ ).

<sup>a,b</sup> Means within the same row with differing superscripts differ ( $P < 0.05$ ).

Least squares means for flow of bacteria, bacterial N and non-microbial N to the small intestine are presented in Table 5. No differences were observed between sources of supplemental protein for flow of bacteria to the small intestine. Similarly, no differences were found between sources of supplemental protein for flow of bacterial or feed N to the small intestine of cows fed diets with SOY, EXP, or FM.

**Table 5.** Least squares means for flow of bacteria, bacterial N and non-microbial N to the small intestine of cows fed diets with soybean meal (SOY), expeller processed soybean meal (EXP), or fish meal (FM) as the source of supplemental protein.

Item	SOY	EXP	FM	SEM
Bacteria, g/d	1756.5	1942.8	1483.6	354.7
Bacterial N				
g/d	160.5	159.0	117.3	43.6
% of N intake	64.5	72.9	50.7	15.2
% of duodenal flow	86.6	86.3	58.4	19.6
Non-microbial N				
g/d	13.3	22.8	80.7	35.4
% of N intake	7.8	19.9	34.6	15.1
% of duodenal flow	13.4	13.7	41.6	19.6

## CHAPTER 4: DISCUSSION

The NRC (2001) recommended a minimum of 12% CP in diets to maintain ruminal fermentation. Although they were formulated to contain 16% CP, diets in this study approached this minimum. The SOY and FM diets used in this study were those used by Richardel (2004) and therefore it is probable that CP content of the forages changed between formulation and experimentation. Diets in this experiment were higher in content of NDF and ADF and lower in content of CP than those of Richardel (2004) which further suggest that forage quality declined between formulation and experimentation.

Dry matter apparently digested in the rumen for this trial averaged 58% while Martin *et al.* (1999) reported 35% for beef steers fed diets differing in grain source. The data reported by Martin *et al.* (1999) differed from our trial because they fed high concentrate diets with wheat, flint, or dent corn. They reported that in steers fed the diet that contained wheat, DM apparently digested in the rumen was 49% of DMI which was similar to our trial. However, the two types of corn were reported to be much lower in DM (dent corn 32% and flint corn 25%) apparently digested in the rumen. In this experiment, apparent total tract digestibility of DM averaged 77% and was not affected by treatment. This agrees with the data of Richardel (2004) who reported 73% total tract DM digestibility in Holstein steers fed diets with SOY or FM and with Ipharraquerre *et al.* (2005) who reported 66% total tract DM digestibility in Holstein heifers fed low crude protein diets (14% CP) with SBM and a blend of animal-marine protein supplements plus ruminally protected Met.



Similarly, OM digestibility in this study also agrees with previous reports. Apparent OM digestibility in the rumen averaged 63% in this study while, Yang *et al.* (2002) reported 79% of OM apparently was digested in the rumen of dairy cows fed alfalfa with varying ratios of alfalfa silage to hay. Apparent total tract OM digestibility in this trial averaged 79% which was 7% higher than that reported by Richardel (2004). Koenig *et al.* (2003) reported 66% OM truly digested in the rumen for cattle fed diets varying in the degree of barley grain processing with 20% barley silage. Organic matter truly digested in the rumen averaged 77% in this trial while Ipharraquerre *et al.* (2005) reported only 38%. Ipharraquerre *et al.* (2005) used lactating Holstein cows consuming approximately 2.5 fold greater DM which would increase passage rate and decrease the amount of nutrients digested in the rumen or total tract (NRC, 2001).

The increased intake of DM and OM when FM was included as a source of supplemental protein is probably related to the increased ruminal and total tract digestibilities of those nutrients. Increased digestibility of diets allow for greater intake by reducing gut fill and decreasing the amount of undigested feed residues that must be excreted daily. Fish meal also is high in RUP content and has a good amino acid balance (NRC, 2001). Including FM in diets in this study may have also improved intestinal AA profiles and stimulated intake because of a general improvement in nutritional status.

Data in this study agree with those of Donaldson *et al.* (1991) who reported an increase in total tract DM digestion in 12 month old Hereford steers grazing annual ryegrass supplemented with FM and distiller's dried grains compared to animals supplemented with corn. Similarly, England and Gill (1985) reported increases in OM

and N digestibility in British Friesian steers fed ryegrass silage supplemented with FM compared to steers supplemented with sucrose.

Nutrient availability is largely determined by rate and extent of ruminal digestion (Nocek and Russell, 1988). The FM could have influenced ruminal fermentation by providing a slow release of N to match the energy availability from the fiber. Diets with FM have slower ruminal protein degradability than those with SOY due to the larger amount of RUP in the FM. Therefore FM combined with the slow degradability of hay makes this a slow carbohydrate-slow protein diet which should be more effective in producing greater energy status and higher nutrient availability than diets with mismatched rates of protein and carbohydrate degradability. In diets with matched rates of protein and carbohydrate degradability, virtually all ruminally degraded CP could have been used for microbial growth (Nocek and Russell, 1988). With diets that have mismatched rates of protein and carbohydrate degradability (hay and SOY), there probably was a surplus of CP available in the rumen and resulted in excess protein degradation and deamination.

Neutral detergent fiber apparently digested in the rumen in this study averaged 66.8% while Ipharraguerre *et al.* (2005) reported 40% and Koenig *et al.* (2003) reported 42%. Data reported by Ipharraguerre *et al.* (2005) differed from our trial because they used high producing dairy cows consuming greater amounts of feed. Similarly, Koenig *et al.* (2003) reported higher apparent rumen digestion of NDF because their diets included two levels of forage and two degrees of barley grain processing with dietary CP levels of 14%. Our results were similar to those by Donaldson *et al.* (1991) who reported 73% NDF digestion in the rumen for steers supplemented with ruminal escape protein

and grazing annual ryegrass. Neutral detergent fiber apparently digested in the total tract was observed to be 80% of intake in this trial, while Richardel (2004) reported 54%, Ipharraguerre *et al.* (2005) reported 42% and Koenig *et al.* (2003) reported 64%. Acid detergent fiber apparently digested in the rumen averaged 69% in this study while Ipharraguerre *et al.* (2005) reported 42% and Koenig *et al.* (2003) reported 20% for ADF apparently digested in the rumen. Acid detergent fiber apparently digested in the total tract averaged 72% in this trial while Richardel (2004) reported 68%, Ipharraguerre *et al.* (2005) reported 36% and Koenig *et al.* (2003) reported 40%.

Nitrogen apparently digested in the rumen in this trial averaged 19% which was low compared to 47% reported by Yang *et al.* (2002). On the other hand, N apparently digested in the total tract for this trial was similar to other previous observations. We observed an average of 64%, while Richardel (2004) reported 79% and Koenig *et al.* (2003) reported 77%. Similarly, our research mirrored others for N apparently digested postruminally for percentage of intake and percentage of duodenal passage. Apparent postruminal N digestion was 42% of intake while Ipharraguerre *et al.* (2005) reported 65% and Koenig *et al.* (2003) reported 76%. As well, N apparently digested postruminally for % of duodenal passage was 55% while Ipharraguerre *et al.* (2005) reported 64% and Koenig *et al.* (2003) reported 77%.

Bacterial N as a % of intake was highest for SOY (65%) and lowest for FM (51%). Similarly to SOY, Koenig *et al.* (2003) reported 70% bacterial N. Yang *et al.* (2002) reported bacterial N of 43% in diets containing alfalfa which was closer to our FM observation. Non-microbial N as a % of intake was lowest for SOY (8%) and highest for FM (35%). Koenig *et al.* (2003) reported 28% and Ipharraguerre *et al.* (2005) reported

45% for non-microbial N. Protein sources used in this trial were chosen to provide a relatively large range in ruminal degradability. The shift in duodenal protein composition from mostly microbial to mostly non-microbial as protein source changed from SOY to FM appears to indicate that there was a wide range in ruminal protein degradability.

Soybean meal is the “Gold Standard” by which almost all other protein sources for livestock are compared (Drackley, 2000). Fish meal and EXP are commercially available protein sources that are considered to be less ruminally degradable than SOY and have a good AA profile (NRC, 2001). In this trial cows fed FM had the greatest daily intake of N while those fed SOY had the lowest intake of N. This is due to the combination of greater DMI when cows were fed FM and a numerically larger concentration of CP in the FM diet. In a summary of studies, Santos *et al.* (1998) reported that nitrogen intake was generally not affected by protein source. These researchers indicated that feeding high RUP sources resulted in a numerical increase in N intake in 12 comparisons and a decrease in 13 comparisons. In the present study, cows fed SOY had the greatest digestion of N in the rumen while those fed FM had the lowest. However, total tract N digestion was similar for all three protein sources. Nitrogen apparently digested postruminally was highest for FM and lowest for SOY.

Davenport *et al.* (1995) reported an increase in nitrogen retention and blood urea nitrogen when dietary nitrogen increased in Suffolk whether lambs fed increasing amounts of dietary nitrogen (9, 12, or 15% CP). Bunting *et al.* (1989) reported an increase in nitrogen retention in Angus heifer calves with high protein intake (18.8% CP) compared to calves with low protein (10.2). In this trial, cows fed FM consumed more N than those fed the other protein sources. They also had the highest total tract N

digestibility of the three protein sources. Therefore, data from this study tend to agree with those of Davenport *et al.* (1995) and Bunting *et al.* (1989) in that cows consuming larger amounts of N appear to digest more of that N and presumably have it available for productive functions. It is not surprising that no statistical differences in N digestion were observed due to increased N intake in this study by cows fed FM. The studies of Davenport *et al.* (1995) and Bunting *et al.* (1989) both used growing animals, while non-lactating, mature cows were used in this trial. Because there was not a large production requirement for these cows, it was highly unlikely that they would exhibit any preference for N source toward growth.

## CHAPTER 5: SUMMARY AND CONCLUSIONS

### Summary

The objective of this study was to determine if substituting protein sources with decreased ruminal degradability for soybean meal in diets fed to non-lactating Holstein cows would alter nutrient digestibility and flow through various segments of the gastrointestinal tract.

Six non-lactating Holstein cows (average BWT 642 kg) were surgically fitted with cannulas in their rumen and proximal duodenum. These cows were then used in a replicated  $3 \times 3$  Latin square designed experiment. Due to factors unrelated to the trial, one cow died during the first period of the study and was not replaced. Supplemental protein was provided by soybean meal (**SOY**), expeller processed soybean meal (**EXP**; West Central Cooperative), or menhaden fish meal (**FM**). Basal diets consisted of (DM basis) 33% corn silage, 20% bermudagrass hay, 27% ground corn, and 2% minerals and vitamins. Supplemental protein was provided as (DM % of total diet) 18% soybean meal (**SOY**), 17% soybean meal and 1% fish meal (**FM**), or 12% soybean meal and 6% expeller processed soybean meal (**EXP**). Diets were offered once daily in amounts adequate to allow for 10% (as offered) feed refusals. Period length for this experiment was 14 days long, which included a 10-d adjustment period and 4-d sample collection period. Two gelatin capsules containing 10 g of  $\text{Cr}_2\text{O}_3$  were placed in the rumen once daily beginning on day 10 of each period to be used as an external marker of digesta flow. Samples of duodenal contents, ruminal contents, ruminal fluid, and feces were collected starting at 0800 h on day 12 of each period and every 3 h thereafter with sampling time being advanced 2 h on each day. Therefore, samples were collected that represent each

hour on the 24 h clock. Diets and refusals were sampled on each sampling day of each period.

Concentration of CP in this study was low and may have been marginal for optimal ruminal fermentation. Including FM as a source of supplemental protein increased DMI and OMI in non-lactating cows. In addition, using FM tended to increase the amount of DM and OM digested in the total tract and the amount of OM apparently digested in the rumen. Using unprocessed soybean meal as the source of supplemental protein decreased N intake and the amount of N digested in the total tract. Meanwhile, source of supplemental protein had no effect on apparent digestion of N in the rumen or postruminally. During this study it was also determined that source of supplemental protein had no effect on nutrient digestibility in any segment of the gastrointestinal tract. Additionally, source of supplemental protein had minor effects on nutrient intake and amount digested in non-lactating Holstein cows.

## **Conclusions**

When formulating diets for non-lactating cows, careful consideration of protein source may allow for lower inclusion rates with minimal impact on diet digestibility and ability to support the maintenance functions of the cow. Feeding low ruminal degradable protein to non-lactating cows resulted in no appreciable impact on feed intake or apparent diet digestibility.

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## **VITA**

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